Research Article

EASL EUROPEAN ASSOCIATION FOR THE STUDY OF THE LIVER | JOURNAL OF HEPATOLOGY

Genetic associations in Italian primary sclerosing cholangitis: Heterogeneity across Europe defines a critical role for *HLA-C*

Johannes R. Hov[1,2,3,†], Ana Lleo[4,5,†], Carlo Selmi[4,5], Bente Woldseth[1,2], Luca Fabris[6,7], Mario Strazzabosco[8,9], Tom H. Karlsen[1,3,*], Pietro Invernizzi[5]

[1]*Department of Medicine and Research Institute for Internal Medicine, Rikshospitalet, Oslo University Hospital, Oslo, Norway;* [2]*Institute of Immunology, Rikshospitalet, Oslo University Hospital, Oslo, Norway;* [3]*Faculty Division Rikshospitalet, Faculty of Medicine, University of Oslo, Oslo, Norway;* [4]*Department of Translational Medicine, Università degli Studi di Milano, Rozzano, Italy;* [5]*Division of Internal Medicine and Hepatobiliary Immunopathology Unit, IRCCS-Istituto Clinico Humanitas, Rozzano, Italy;* [6]*Department of Surgical and Gastroenterological Sciences, University of Padova, Padova, Italy;* [7]*Center for Liver Research (CeLiveR), Ospedali Riuniti di Bergamo, Bergamo, Italy;* [8]*Department of Clinical Medicine and Prevention, University of Milan-Bicocca, Monza, Italy;* [9]*Liver Center, Yale University, New Haven, USA*

Background & Aims: The HLA complex on chromosome *6p21* is firmly established as a risk locus for primary sclerosing cholangitis (PSC). We aimed to exploit genetic differences between Northern Europe and Italy in an attempt to define a causative locus in this genetic region.

Methods: Seventy-eight North-Italian PSC patients and 79 controls were included. We performed sequencing-based genotyping of *HLA-C*, *HLA-B*, and *HLA-DRB1*. The major histocompatibility chain-related A (*MICA*) transmembrane microsatellite was analysed using PCR fragment length determination. The tumour necrosis factor-alpha (*TNF-α*)-308G→A polymorphism was genotyped with TaqMan®. Allele frequencies were compared with Chi-square tests. Uncorrected *p*-values <0.05 were considered statistically significant when replicating findings in previous studies. The *p*-values of novel associations were corrected for multiple comparisons (Bonferroni).

Results: The frequency of the strong inhibitory HLA-C2 killer-immunoglobulin receptor (KIR) ligand variant was significantly reduced in PSC *vs.* controls (0.39 *vs.* 0.58, $p = 0.0006$). Consequently, HLA-C1 homozygosity was associated with an increased risk of PSC (OR 3.1; 95% CI 1.4–6.7, $p = 0.004$). Importantly, there were no significant associations with the HLA-Bw4 KIR ligand variant, at the neighbouring *MICA* locus or with TNF-α-308G→A. At *HLA-DRB1*, we confirmed positive and negative associations with DRB1∗15 and DRB1∗07, respectively, while there were no associations with the DRB1∗03, ∗04 or ∗1301 alleles typically detected in PSC in Northern Europe.

Conclusions: The strong inhibitory of the KIR ligand HLA-C2 protects against PSC development in all populations hitherto studied. Further studies on the role of natural killer cells and T-lymphocytes expressing KIRs in PSC pathogenesis are warranted.

Keywords: Primary sclerosing cholangitis; HLA; Natural killer cells; Killer immunoglobulin-like receptors.

Received 21 August 2009; received in revised form 15 October 2009; accepted 1 November 2009; available online 4 March 2010

*Corresponding author. Address: Norwegian PSC Research Center, Department of Medicine, Rikshospitalet, Oslo University Hospital, Oslo N-0027, Norway. Tel.: +47 23 07 2469; fax: +47 23 07 3510.

E-mail address: t.h.karlsen@medisin.uio.no (T.H. Karlsen).

†These authors contributed equally to this work.

Abbreviations: PSC, primary sclerosing cholangitis; HLA, human leukocyte antigen; MICA, major histocompatibility complex class I chain-related A; TNF-α, tumour necrosis factor-alpha; KIR, killer immunoglobulin-like receptor; NK cells, natural killer cells; LD, linkage disequilibrium; PCR, polymerase chain reaction; SNP, single-nucleotide polymorphism; SSO, sequence-specific oligonucleotides; SSP, sequence-specific primers; OR, odds ratio.

Introduction

The aetiology of primary sclerosing cholangitis (PSC) is unknown [1]. Siblings have a 9-39x increased risk of PSC as compared to the overall population, suggesting the presence of a heritable component in the pathogenesis [2]. Genetic variation in the large HLA complex at chromosome *6p21* influences PSC susceptibility, but the exact disease genes have not been possible to define [3].

Since the 1982 report on an HLA-B8 and DR3 association [4], the main focus of genetic studies in PSC has been the HLA class I (mainly *HLA-A*, *-B* and *-C*) and class II (mainly *HLA-DRB1*, *DRB3*, *DRB4*, *DRB5*, *DQA*, and *DQB1*) genes, which encode molecules involved in antigen presentation to T cell receptors [5]. In addition to HLA-B∗08 and DRB1∗03 (serologic B8 and DR3), the main HLA gene variants associated with PSC susceptibility are HLA-Cw∗07, a series of *DRB1* alleles (DRB1∗04, ∗07, ∗1301 and ∗15) and corresponding *DRB3*, *DRB5*, *DQA*, and *DQB1* variants [6–11]. These associations could indicate that immune responses against specific (auto-) antigens are pathogenetically important in PSC. However, other genes in the HLA complex (e.g., major histocompatibility complex class I chain–related A [*MICA*] and tumour necrosis factor-alpha [*TNF-α*]) have also been implicated

Table 1. Characteristics of the patients with primary sclerosing cholangitis and the healthy controls.

Features	Patients (n = 78)	Controls (n = 79)
General		
Male sex, n (%)	43 (55)	48 (61)
Age (years)	51	41
median (range)	16–80	18–60
Duration of disease (years)	11	–
median (range)	3–35	
Mayo risk score	0.5	–
median (range)	0.2–1.2	
Clinical		
Inflammatory bowel disease, n (%)	57 (73)[a]	–
Varices, n (%)	7 (9)	–
Cirrhosis, n (%)	13 (17)	–
Cholangiocarcinoma, n (%)	1 (1.2)	–
Laboratory		
Alkaline phosphatase,[b] (U/L)	457	121
median (range)	91–1725	97–270
Aspartate aminotransferase,[c] (U/L)	97	32
median (range)	11–423	21–43
Total bilirubin,[d] (mg/dl)	1.31	0.5
median (range)	0.2–8.0	0.1–1.1
Serum ANCA – positive, n (%)	19 (24)	–

[a] Colonoscopy not performed in six patients. If excluding these, 57 out of 72 (79%) patients had colitis.
[b] Reference value <279.
[c] Reference value <50.
[d] Reference value <1.0.

[12–15]. Recently, it was proposed that the associations at *HLA-C* and *HLA-B* in PSC might result from the variations of these molecules critical to their additional function as ligands for killer immunoglobulin-like receptors (KIRs) on natural killer (NK) cells and various T-lymphocytes [16].

Studies of HLA associations in any disease are complicated by a strong tendency of variants in more than 250 protein coding genes in this region to be inherited together (strong linkage disequilibrium [LD]). There is a considerable genetic variation in the HLA complex along a North-South axis of Europe [17]. Patterns of LD may also vary depending on the study population. Such phenomena may be exploited in attempts to pinpoint the exact genes responsible for disease associations [18]. Two previous studies have assessed HLA associations in Italian PSC [9,11]. None of these have assessed genetic variation within the HLA class I KIR binding motifs or at *MICA*. In the present work, we aimed to systematically re-evaluate HLA class I and II associations in an Italian PSC cohort with a particular emphasis on genetic variation in the vicinity of *HLA-B* and *MICA*.

Materials and methods

Subjects

Seventy-eight PSC patients and 79 healthy controls from Northern Italy were included (Table 1). Diagnosis was based on clinical, cholangiographic and histo-

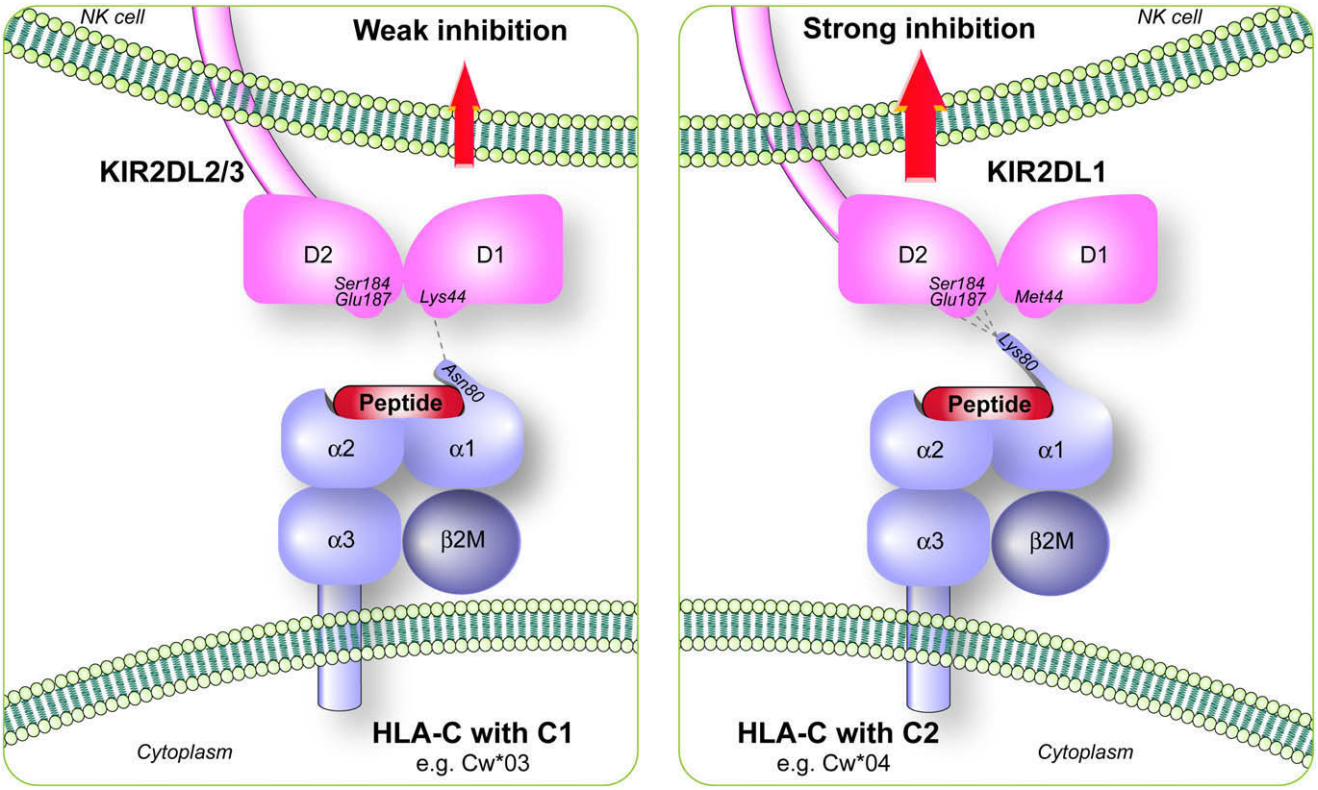

Fig. 1. The interaction between HLA-C1 and KIR2DL2/3 and between HLA-C2 and KIR2DL1. The residue at position 80 of *HLA-C* defines the specificity; C1 carries Asn, while C2 carries Lys [20]. The C1-KIR2DL2/3 interaction gives a weaker inhibition of natural killer cells compared with the C2-KIR2DL1 interaction [32]. **α1/2/3:** subdomains of HLA class I α-chain; **β2M:** β_2-microglobulin; **D1/2:** subdomains of KIRs; **KIR2DL1/2/3:** KIR variants. *Abbreviations:* Asn, asparagine; Lys, lysine; KIR, killer immunoglobulin-like receptor.